Example 9. Comparing ProteoMiner™ Method with Limited Digestion Method

[0096] The efficiencies of native digestion and ProteoMiner™ methods for HCP enrichments were tested and compared using mAb5 containing HCP impurities. As shown in FIG. **7**, a total of 63 HCPs were identified with high confidence using the ProteoMiner™ method of the present application. A total of 25 HCPs were identified with high confidence using the native digestion method. As shown in FIG. **8**, in comparing the ProteoMiner™ and native digestion methods, 21 HCPs were identified by both methods. In comparing the ProteoMiner™ and native digestion methods, 26 HCPs were identified by both methods. Table 14 shows the 63 HCPs which were found in mAb5 sample using ProteoMiner™ method of the present application.

TABLE 14

HCPs found in mAb5 sample.

Accession No.	Protein Name
G3I6T1	Putative phospholipase B-like 2
G3HXN7	Beta-hexosaminidase
G3HLX3	Alpha-N-acetylglucosaminidase
Q9JKY1	Peroxiredoxin-1
G3I4W7	Cathepsin D
G3H892	Aminoacylase-1A
G3I255	L-lactate dehydrogenase
G3GZB2	Acid ceramidase
G3H533	Peptidyl-prolyl cis-trans isomerase
G3IBF4	Serine protease HTRA1
G3I3N5	V-type proton ATPase subunit C
G3I2K6	Hippocalcin-like protein 1
G3GR64	Inter-alpha-trypsin inhibitor heavy chain H5
G3GRS9	N-acetylgalactosamine-6-sulfatase
G3IBH0	Metalloproteinase inhibitor 1
G3I3Y6	Glutathione S-transferase P
G3I1V3	Fibronectin
A0A061IFE2	Liver carboxylesterase 1-like protein
G3GUR1	Complement C1r-A subcomponent
G3GXZ0	Protein-glutamine gamma-glutamyltransferase 2
G3I8R9	78 kDa glucose-regulated protein
G3HGW6	Laminin subunit alpha-5
G3I7U9	Serine protease HTRA2, mitochondrial
G3GVH3	Uncharacterized protein C17orf39
A0A061IQB8	Ubiquitin-60S ribosomal protein L40-like isoform 2
G3IAQ0	Alpha-enolase
G3IFA9	Transcription elongation factor B polypeptide 2
G3II12	Calcium-binding protein 39
G3I3K5	G-protein coupled receptor 56
G3I5N6	Insulin-like growth factor-binding protein 4
G3HXL1	Poly(RC)-binding protein 1
G3HH30	Aldose reductase
Q9EPP7	Cathepsin Z
Q9WV24	Beta-2-microglobulin
A0A061HWZ7	Exosome complex component RRP46-like protein
A0A061II04	Protein S100
G3HI03	U4/U6 small nuclear ribonucleoprotein Prp4
A0A061IB69	Fructose-bisphosphate aldolase
G3I5L3	Annexin
A0A061IDC7	Sp110 nuclear body protein
G3H935	Tyrosine--tRNA ligase
A0A061I0W7	Brain-specific serine protease 4-like protein
G3IEU2	Protein DJ-1
P22629	Streptavidin
A0A061IMN7	Anionic trypsin-2-like protein
A0A061IK25	Protein-L-isoaspartate
A0A061INB9	C-X-C motif chemokine
A0A061I4J0	Prefoldin subunit 2-like protein
G3IG05	Annexin
A0A061I1Y4	Perilipin-4-like protein
A0A061IEQ5	Sphingomyelin phosphodiesterase
A0A061IKI0	EF-HAND 2 containing protein

TABLE 14-continued

HCPs found in mAb5 sample.

Accession No.	Protein Name
A0A061I523	Procollagen C-endopeptidase enhancer 1
G3HIM4	Cell division control protein 42-like
G3H2A5	Vacuolar protein sorting-associated protein 29
G3GV64	Mammalian ependymin-related protein 1
G3HPZ5	Macrophage-capping protein
G3HD94	Desmoplakin
G3HAN8	Adenosylhomocysteinase
A0A061I2S4	Putative out at first protein like protein (Fragment)
A0A061HXN7	Gelsolin
G3HN65	Ras suppressor protein 1
G3HH39	Elongation factor 1-alpha 1
G3IKC3 *	Glutathione S-transferase Mu 6
G3GVW2 *	Putative hydrolase RBBP9
G3GTT2 *	C-C motif chemokine
G3I4E8 *	Fatty acid-binding protein, adipocyte

What is claimed is:

1. A method of identifying host cell protein (HCP) impurities in a sample, comprising:

contacting the sample to solid support,

wherein said sample includes at least one high-abundance peptide or protein,

wherein interacting peptide ligands have been attached to said solid support, and

wherein said HCP impurities can bind to the interacting peptide ligands;

washing said solid support using a solution comprising a surfactant providing an eluent;

subjecting said eluent with an enzymatic digestion condition to generate components of isolated HCP impurities; and

identifying components of the isolated HCP impurities using a mass spectrometer.

2. The method of claim **1**, wherein the surfactant is a phase transfer surfactant, an ionic surfactant, an anionic surfactant, a cationic surfactant, or combinations thereof.

3. The method of claim **1**, wherein the surfactant is sodium deoxycholate, sodium lauryl sulfate, or sodium dodecylbenzene sulphonate.

4. The method of claim **1**, wherein a concentration of the at least one high-abundance peptide or protein is about at least 1000 times, 10,000 times, 100,000 times or 1,000,000 times higher than a concentration of the each HCP impurity.

5. The method of claim **1**, wherein the interacting peptide ligands are a library of combinatorial hexapeptide ligands.

6. The method of claim **1**, wherein the HCP impurities are quantified using the mass spectrometer, wherein a detection limit of the each HCP impurity is about 0.05-0.1 ppm.

7. The method of claim **1**, wherein the at least one high-abundance peptide or protein is an antibody, a bispecific antibody, an antibody fragment, a Fab region of an antibody, an antibody-drug conjugate, a fusion protein, a protein pharmaceutical product, or a drug.

8. The method of claim **1**, wherein an enzyme of the enzymatic digestion reaction is trypsin.

9. The method of claim **1**, wherein the mass spectrometer is an electrospray ionization mass spectrometer, nano-electrospray ionization mass spectrometer, or a triple quadrupole mass spectrometer, wherein the mass spectrometer is coupled to a liquid chromatography system.

10. The method of claim **1**, wherein the mass spectrometer is capable of performing LC-MS (liquid chromatography-